VITAMIN STABILIZATION

Effect of Added Stabilized Animal Fats On Stability of Vitamin A in Feeds

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This work was initiated to determine the stability of vitamin A (as fish liver oil) in mixed feeds with either no added fat or 6% stabilized animal fat added, when the feeds were stored at room temperature. Much interest has developed in the use of animal fats, stabilized with antioxidants, in commercial feeds. The vitamin A stability of the ration was increased when 6% stabilized choice white grease (pork fat) was added to the ration. This increase was noted primarily in the later phases of the storage periods (after 4 to 12 months' storage), and was observed in two series of experiments conducted in two different years. In addition to increased feed efficiency shown with dogs, chicks, and other animals, decreased dustiness of the feed, ease and speed of pelleting of the rations, and improved appearance associated with the addition of stabilized animal fats to feeds, increased vitamin A stability also was observed. Improving the stability of nutrients such as vitamin A during storage of feeds is of importance to all aspects of our agricultural economy.

EVALUATION OF THE EFFECTS OF ANTIOXIDANT-TREATED FAT on the stability of the vitamin A in the feed was important in the course of studies on the effect of adding animal fat, stabilized with antioxidants, on the growth rate and food and caloric utilization by dogs and chicks (*11*, *12*). Correlative studies on comparable feeds with stabilized fat added have shown that the added fat does not become rancid after at least 1 year's storage at room temperature (*8*).

Buxton (*1*), Dyme *et al.* (*3*), and Sandell (*9*) have shown that the vitamin A destruction generally parallels the rise in peroxide value of the vitamin carrier. Increased stability of vitamin A when antioxidants have been added to the system has been observed by a number of workers (*4*, *5*, *13*, *14*). The present study has investigated the effect of stabilized animal fat added to dry dog meals on the stability of vitamin A during room temperature storage.

Experimental Work

The experimental feed used in these studies was a dry dog meal designed to be similar in composition to commercial dry meals. Its composition is shown in Table I. The vitamin A source added to this meal was a commercial fish liver oil (Nopco XX, 2250 U.S.P. units of Vitamin A per gram) which was blended either directly into the meal (control meal) or dissolved previous to blending in a weighed amount of stabilized fat equal to 6% of the weight of the dry meal. Vitamin A analysis of random samples of the meals indicated a very uniform distribution of the vitamin throughout.

The animal fat (choice white grease, a high grade of inedible pork fat) was stabilized by the addition of butylated hydroxyanisole (0.02%), citric acid (0.01%), and propyl gallate (0.005%) to the melted fat at 80° C. (*2*, *6*). The choice white grease had an initial peroxide value of 9, a free fatty acid content of 2.9%, FAC color (method of Fats Analysis Committee, American Oil Chemists' Society) of 13, and a titer of 39.3° C.

The experimental meals (either with or without 6% fat added at the expense of the entire ration) were stored in 1-pound glassine-lined paper bags at room temperature (approximately 78° F.). Two series of duplicate samples were stored for approximately 1 year and assayed for vitamin A at intervals throughout the storage period. The first series was made up in May 1951; the second series was made up and storage tests were initiated in May 1952.

The chromatographic method for the determination of vitamin A in mixed feeds as reported by Schaeffer (*10*) was used for the determination of vitamin A in these studies. This method consists of a petroleum ether (Skellysolve B fraction) extraction of a 30-gram sample of the feed under reflux for 1 hour, partial evaporation (vacuo) of an aliquot, adsorption on a 1 to 1 magnesia–Filter Cel column, elution with 10% acetone in petroleum ether, and total evaporation (vacuo) of the eluate; the residue was taken up in chloroform and an aliquot was analyzed, using the Carr-Price colorimetric reaction with 25% antimony trichloride. Color intensity was measured with a Coleman Jr. spectrophotometer at 620 mμ.

As vitamin A was being determined on crude feed samples, it was important to

Table I. Composition of Experimental Ration

Ingredient	%
Corn flakes	26.75
Wheat flakes	26.70
Soybean grits (HI-PRO-CON)	19.00
Meat and bone scrap	15.00
Fish meal (menhaden)	3.00
Wheat germ meal (defatted)	5.00
Dried skim milk	2.50
A & D oil (Nopco XX, 2250 U.S.P. units A, 400 AOAC units D/g.)	0.50
Iodized salt	0.25
Brewer's yeast (nondebittered)	0.50
Riboflavin supplement (BY-500)	0.80
	100.00

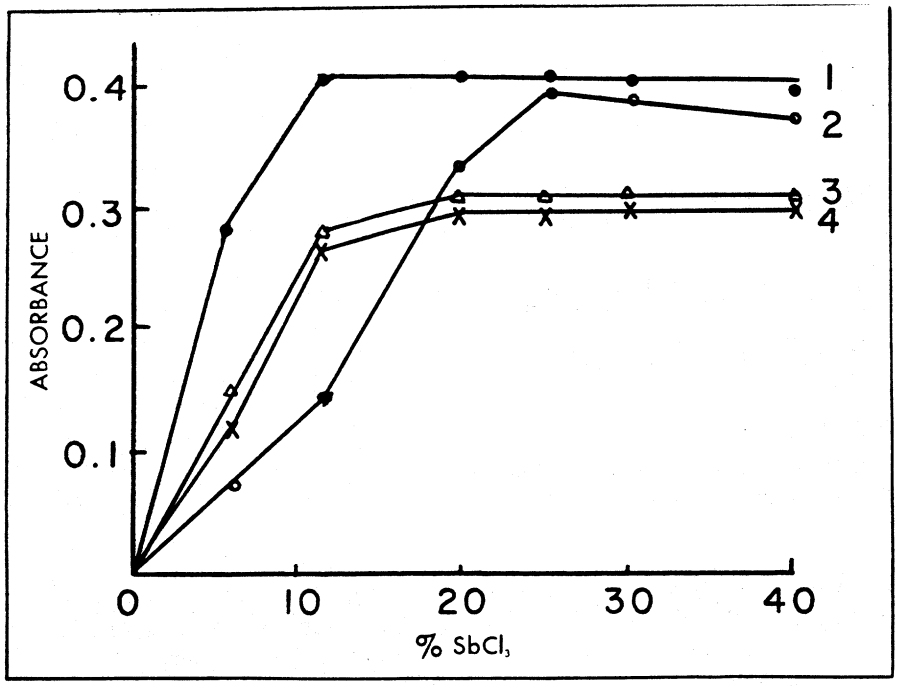


Figure 1. Effect of increasing concentrations of antimony trichloride on absorbance observed at 620 mμ with addition of vitamin A

1. Vitamin A reference oil
2. Fish liver oil concentrate
3. Extract of basal ration
4. Extract of basal ration with 6% stabilized fat added

determine the concentration of antimony trichloride required for maximum color development. Figure 1 shows the results of varying the antimony trichloride concentration on the color intensity at 620 mμ using the various vitamin A sources. Maximum color intensity was obtained from the reference standard oil at 10% antimony trichloride concentration, while 25% antimony trichloride was required for maximum color intensity with the fish liver oil concentrate and the experimental feed extract. In view of these observations, 25% antimony trichloride was used for all subsequent analyses.

Carotene interference was negligible in these studies, owing to the extremely low carotenoid content of the experimental meal.

U. S. P. vitamin A reference standard oil (vitamin A acetate in cottonseed oil, 1 gram equals 10,000 U.S.P. units of vitamin A alcohol) was used in the determinations of the standard curves for the colorimetric assays of vitamin A and also for all internal standards employed during the assays.

The results obtained in each of the two storage tests (series I and II) are shown graphically in Figures 2 to 5 (the vitamin A content of the meal containing 6% stabilized fat has been calculated on the basis of dry weight of the control meal by correcting for the dilution due to the added fat).

The individual assays of duplicate series of meals were in excellent agreement and recoveries of internal standards and comparison with a saponification procedure (7) indicate that the method of determining vitamin A was satisfactory. Some typical recoveries of added internal standard are shown in Table II.

The results for the stored samples of series I through the first 12 weeks' storage (Figures 2 and 3) show that the vitamin A in the meal containing 6% stabilized fat was as stable as the vitamin A contained in the control meal. In the later phases of the storage period, vitamin A in the sample that contained added fat was considerably more stable than in the control meal for both sets of samples. The retention of vitamin A through the first 18 weeks' storage was approximately 50 to 60% of the original vitamin A values for both experimental meals. The vitamin A potency in the meal containing the stabilized fat remained at this level throughout the remainder of the storage period, whereas the vitamin A values of the control meal declined to approximately 30 to 40% of the original values.

Similar results were obtained from samples of series II studied in the second year (Figures 4 and 5). However, the over-all stability of vitamin A during this storage period is somewhat higher than the stability noted in the series I samples (Figures 2 and 3). The meal containing 6% stabilized fat again showed somewhat more stabilization of the vitamin A than the control meal and

Figure 2. Effect of adding 6% stabilized fat on vitamin A potency of feeds stored at room temperature

Series I, 1951
1. Basal ration
2. Basal ration + 6% stabilized fat

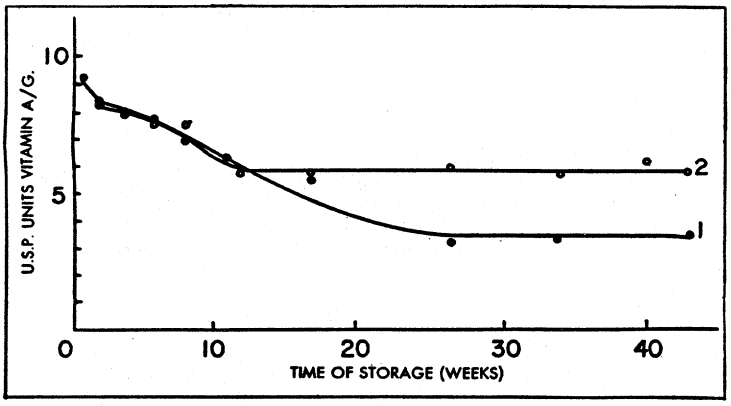
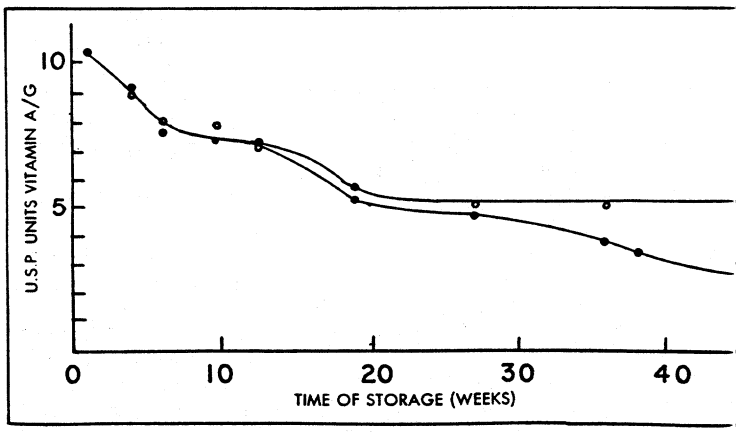


Figure 3. Effect of adding 6% stabilized fat on vitamin A potency of feeds stored at room temperature

Series I, 1951
1. Basal ration
2. Basal ration + 6% stabilized fat



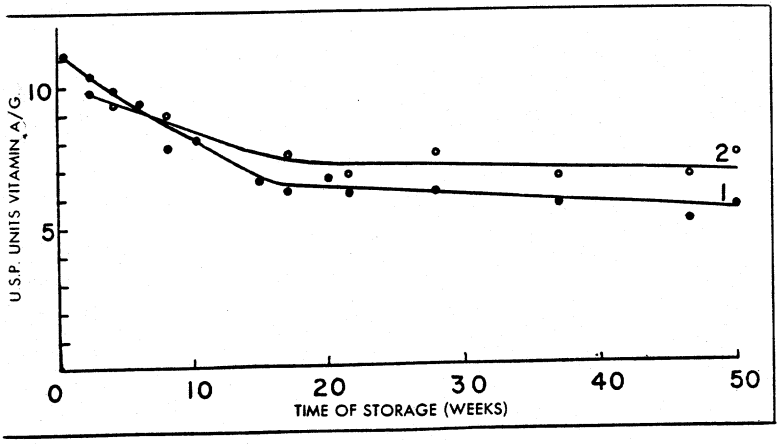


Figure 4. Effect of adding 6% stabilized fat on vitamin A potency of feeds stored at room temperature

Series II, 1952
1. Basal ration
2. Basal ration + 6% stabilized fat

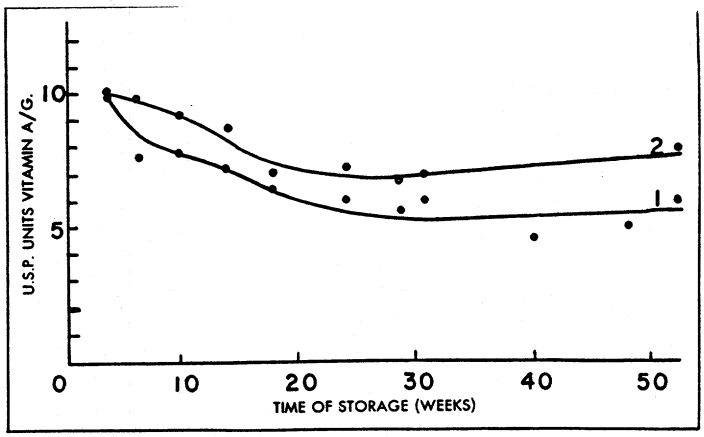


Figure 5. Effect of adding 6% stabilized fat on vitamin A potency of feeds stored at room temperature

Series II, 1952
1. Basal ration
2. Basal ration + 6% stabilized fat

the rate of loss of the vitamin during the first 15 weeks of storage was considerably higher in the control meal.

The results of both experiments show that the addition of 6% stabilized fat to the experimental meal did not decrease the stability of the vitamin A added to the meal as a fish liver oil and may have increased the stability of the vitamin. The apparent increased stability of the vitamin A probably is due to the antioxidants present in the added fat, which afford a direct protection to the vitamin in addition to preventing peroxide formation in the added fat. Peroxide values determined on the fat at various times throughout storage periods up to 47 weeks ranged from 3 to 11. The data (obtained in collaboration with L. R. Dugan and associates) showed no correlation of peroxide values with storage time of the feed.

Summary

Stored samples of feeds used for nutritional studies with dogs, prepared either with or without 6% of antioxidant-treated fat, were analyzed periodically for vitamin A by chemical analysis in order to determine the stability of this vitamin when these meals were stored at room temperature.

The chemical determination of vitamin A in these meals was satisfactory as judged by comparison with a saponification procedure, duplicability and agreement of results, and recoveries of internal standard.

The results of two series of storage experiments (each series approximating a 1-year storage period) show that the addition of 6% stabilized fat to the experimental meal does not decrease the stability of the vitamin A in the meal (added as fish liver oil) and appeared to have increased its stability.

Table II. Recovery of Internal Standard by Chromatographic Procedure for Determination of Vitamin A in Mixed Feed

Sample Description	Vit. A Potency of Internal Standard Added/G.[a]	Vit. A. Potency/G. by Assay[a]	Internal Standard Recovered, %
I. Vitamin A standard			
1	23.5	22.3	95
2	23.5	21.3	91
3	23.5	22.0	94
II. Basal ration			
1	None	8.3	...
2	16.7	24.6	98
3	16.2	24.9	102
III. Basal ration[b]			
1	None	3.4	...
2	8.8	11.8	96
IV. Basal and 6% fat[b]			
1	None	5.9	...
2	9.5	16.3	109

[a] U.S.P. units.
[b] Results on samples stored for 38 weeks.

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